February 18, 1954

The origin and behavior of c-m2; the origin and behavior of Wx-ml.

I. Review of previous discussion.

1. Attempted to show the action of Ac on two different loci it controls when these are located in two different chromosomes in the same nucleus.

a). Both respond alike in any one kernel to doses of Ac -- 1, 2 and 3 Ac.

b). Somatic changes in Ac leading to formation of sector with altered dosage action -- reflected in the mutation or break behavior at both loci.

2. Question would appear: Does Ac control not only the time during development when changes at c-ml or Ds will occur, but also the cell in which it will occur?

a). The test of this: c-ml wx / c-ml wx / c Wx Ds, Ac Ac ac. The sectors in which the Ac action had altered examined to give patches of C and patches of wx. Do these patches coincide? Do they represent events occurring to c-ml and to Ds in the very same cell?

b). How this could be told:

Assume, during development, mutation to c and break at Ds occur in the same cell:

All cells arising from this one would be C in genotype
All cells arising from this one would also be wx in genotype.

Sector would be formed from this cell: The outer part of this sector terminates in the aleurone layer -- C color. Underneath, the starch in the cells of endosperm would stain wx:

Diagram of the sector:

on back

c). Would expect coincidences to appear in high numbers, only if the states were reciprocal in this case -- the state of c-ml must give a very high frequency of mutations to C and few if any breaks. That of Ds must give a high frequency of breaks to eliminate the Wx. Deviations from these two types of behavior would not give a coincident C wx sector.

d). In the test made, a high coincidence of C aleurone with underlying wx starch. This indicates that both c-ml and Ds are altered in the same cell at the same time.

3. Conclusions: The coincidence observed suggests that Ac effects the expression of Ds, wherever it may be located and regardless of numbers, in the same cell at the same time -- within limits difficult to determine.

The time during development and the cells in wich Ac-controlled changes at the loci it controls is not a random event. Some conditions must arise in these cells, under the influence of Ac, that results in changes at both c-ml and at Ds-standard location.

why

II. Review - continued:

1. Attempt made to show that the control of when and in what cells Ds breaks or mutations at c-ml will occur depends upon the state as well as the dose of Ac . Used "stabilized" Ac state as an illustration.

2. General conclusions:

a). Ac controls when and where changes will occur at the various loci it controls -- cm-1, bz-ml, Ds at any location. This by dose of Ac, and by state and dose of Ac.

b). The consequence of a change at an Ac-controlled locus depends on the state of that locus itself:

State 1	(c)	C	(c)	(c)	c
series of cells in which changes occur	x	x	x	x	x
State 2	C	C	(c)	C	C

3. The question of Dr. Lewis: Does the addition of Ds loci to the nucleus have any affect on the action of these loci -- that is, modify Ac action? Answer: No effects have been noted of the addition of Ds to the action of Ac. Appear to be independent.

4. Discussed briefly the origin and behavior of bz-ml. Much like that of c-ml.

5. Began the discussion of c-m2; its origin.

III. Continue account of c-m2, page 3 of Feb. 15 outline.

IV. General conclusions from discussions of c-ml and c-m2.

1. Mutations of c-ml: To original C type action. Intermediate alleles not produced.

2. Mutations of c-m2: Exhibit a variety of phenotypic actions; quantitative and qualitative differences in action exhibited by the various different mutants.

3. What does this mean with regard to the genic action at the normal C locus

mp 4. Does the action of c-m2 indicate the the C locus is compound?

5. If not, what is the nature of the change, or series of changes at the locus of c-m2 that is responsible for the variety of different types of action?

6. How may we interpret genic action at a single locus ? Does some type of control exhist at the locus which alters the type and degree of genic action? Can we explain the behavior of c-m2 on some basis other than that it reflects a compound gene? Is there some better interpretation and how can it be tested?

MP

7. Before attempting to find answers for these questions, it would be better to wait until more of the actions at mutable loci have been considered.

The origin and behavior of wx-ml

I. The origin:

c sh wx ds ac females x $\frac{\text{c-m2 Sh Wx}}{\text{c-m2 Sh Wx}}$ $\frac{\text{Ac}}{\text{Ac}}$ male

Kernels: Nearly all c to C Sh Wx.

One kernels: c-C Sh; wx to Wx. Wx spots in a wx background.

Aleurone layer sliced off and starch in endosperm cells below stained with I-KI solution:

Areas of different intensities of blue staining in a red-staining background.

II. Plant from this kernel grown. Pollen examined:

1. Pollen: How produced; haploid condition; starch in grain; Appearance with wx; Appearance with normal Wx -- deep blue. with I-KI solutions.

Pollen of particular plant: Most grains wx.
Few grains staining blue with I-KI
Intensity of stain varied from pale lavender to intense blue.

Indicated that a mutable wx locus present.

Tests, indicated below, showed that mutable wx carried by the c-m2 Sh chromosome. Origin, then, in the c-m2 Sh Wx parent plant.

2. The crosses of this plant to those that were c sh wx ac:

a). Linkage relations of c to C and wx to Wx kernels indicated the presence of a mutable wx in the c-m2 Sh chromosome.

Constitution of plant: $\frac{\text{c-m2 Sh wx-ml}}{\text{c sh wx}^{s}}$ $\frac{\text{Ac}}{\text{ac}}$

Appearance of wx to Wx in ½ of the kernels, the linkages with the c to C variegated kernels, etc., suggested Ac control of mutations at wx-ml.

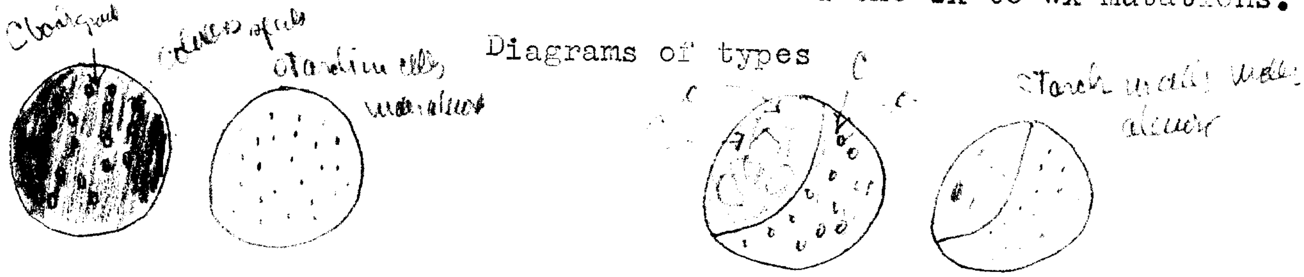
3. The ~~self-pollinated ear~~ crosses by c sh wx ac -- also showed evidence of Ac control through dosage action -- speckled for c to C mutations; spk for wx to Wx; Early mutations of c to C also early mutations of wx to Wx alleles.

4. Plant crossed by pollen from a C Sh wx Ds ac tester plant:

a). wx to Wx, and speckled pattern, only in those kernels that were also C to c variegated due to Ds breaks in C Sh wx Ds chromosome.

b). Sector with changed Ac action -- affected in same manner both the C to c areas (breaks in C Sh wx Ds chromosome) and the wx to Wx mutations.

Diagrams of types



5. Subsequent tests: no doubt about the Ac control of mutations of wx-ml.

– Begin here. Feb. 22

III. The nature of the mutations occurring at wx-ml.

1. The quantitative nature of the action of the normal alleles, Wx and wx.

a). wx wx wx endosperm -- starch is amylopectin. Stains red with I-KI

b). Wx wx wx " 22% of starch is amylose 80 % amylopectin Stains blue with I-KI

c). Wx Wx wx " 25% amylose " " " "

d). Wx Wx Wx " 28% " " " " "

e). Physical appearance of the different kernels above: wx wx wx is like candle wx in appearance; Wx -- more translucent.

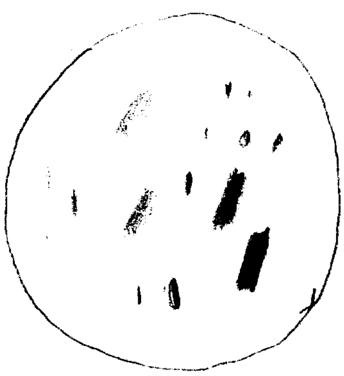
2. the known wx^a mutant from S.A. -- one dose, 0.65% amylose. Stains very faint lavendar. Physical appearance, waxy.

3. Mutants produced by wx-ml. On criteria of both staining and physical appearance, many different levels of production of amylose.

All different types can appear in same endosperm:

c wx/c wx/ c wx-ml 1 Ac:

Diagram of types:



4. The germinal mutat ns. How obtained:

Female x Male

c ~~wx~~ c wx-ml Ac/ac

a). Types of germinal mutations: Whole kernels with mutation to one of the alleles of Wx. - Stain from faint lavendar to very deep blue. Physical appearance follows the staining reaction -- from waxy to very translucent.

b). The low amylose allele: Show faint staining. Majority are associated with altered growth conditions in endosperm -- outer region of endosperm seems [illegible] and wrinkled. This condition not seen in the kernels with the higher alleles of Wx. Condition expressed as a dominant.

b). In above cross, two types of kernels with respect to stabilities : those that show no change in staining throughout the kernel and those with areas of altered staining reactions -- wx, light lavendar or dark blue.

c). The t~~y~~wo types associated with presence and absence of Ac. Stability of mutant when Ac absent; When Ac present, further mutations occur, alt ough, as with the pinks from c-m2, the rate much reduced over wx-ml or c-m2.

5. Conclusions:

(1). When Ac present, mutations at wx-ml to various alleles of Wx -- quantitatively expressed. When Ac absent, no change at the locus.

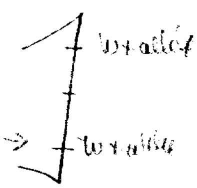
(2). Germinal mutations occur to various alleles. These stable in absence of Ac but unstable when Ac present.

(3). Behavior of wx-ml quite similar to c-m2 in essentials: quantitative expressions; two types of mutants; relative stability of the germinal mutants with Ac in comparison with either original, that is, c-m2 or wx-ml rates.

6. The additive effects of doses of the mutants with low amlyose production.

a). Breakage-fusion-bridge cycle:

Female, wx^S x male, chromosome 9 with broken end and low allele of Wx from wx-ml:



b). The dosage action of the lower alleles in 1, 2 and 3 doses: No Ac:

2 The change in physical appearance with increased doses:

wx wx wx

Wx,allele, wx wx
Wx-allele, Wx-allele, wx
Wx-allele, Wx-allele, Wx-allele

c). Chemical analyses of amylose content of the various alleles and doses of them: (Made by Sprague and Brimhall, Iowa State Univ.)

% amylose starch

Wx	1 dose	2 doses	3 doses
4744A-3	2.4	4.0	5.8
5220A	3.5	4.6	7.3
4744E-1	3.7	4.6	7.2
4744E-2	3.7	5.8	*
2nd sample		5.3	
4744G-2	4.3	6.5	*
5013D	4.6	5.0	9.3
4742B-2	6.5	7.3	13.2
4721F	16.8	22.2	25.0
4744C	18.5	20.0	25.5
5750B	25.0	27.0	28.0
5016A	23.0	27.0	*

* Sample with triple dose not available at time of analysis

IV. General Conclusions:

1. In plants with c-m2 and normal Wx, and Ac, change occurred at Wx locus to produce the wx-m1 condition.
2. Mutations occur when Ac present -- at times dependent upon dose of Ac and its state. No mutations without Ac.
3. The types of mutations in both c-m2 and wx-m1 much alike although the reactions effected obviously not the same -- anthocyanin vs. starch structure.
4. The unit responsible comparable to Ds but not necessarily Ds as in other cases reported. May be similar type of unit, however. Responds to Ac.
5. Two types of mutants at wx-m1. (a) Those altering amount of amylose produced, and those (b) giving a change in the structure of the kernel -- a morphological alteration related to (?).
6. The types of mutations occuring at wx-m1 are not typical for all mutable conditions arising at normal Wx locus. Some behave like c-m1, that is, wx to full Wx; Their origins independent in each case.
7. It is not the mutations of the gene that are being expressed in all these cases (c-m1; c-m2; bz-m1, wx-m1) but changes in the gene controlling materials that are associated with them or become associated with them. These effect how the genic substance at the locus can act. Changes in them control the type of change in genic action/ When and where these changes will occur depends on Ac.